influence the development of aggressive fibromatosis in FAP patients.

In colorectal cancer, obesity and hyperlipidemia are known to increase tumor risk. In our experiments, *Min* mice develop intestinal polyps along with high serum TG levels up to 10-fold those observed in wild-type mice (52). We further found that serum Pai-1 levels in the 15-week-old male *Min* mice were eight times higher than in wild-type mice and hepatic Pai-1 mRNA levels were 11-fold increased. Immunostaining for Pai-1 was also strong in small intestinal epithelial cells of *Min* mice. Thus, it is conceivable that PAI-1 is one of the factors that might explain linkage between hyperlipidemia and intestinal tumorigenesis.

Administration of a PAI-1 inhibitor, SK-216, at 25, 50 and 100 ppm doses in the diet for 9 weeks reduced serum Pai-1 levels and hepatic Pai-1 mRNA levels of *Min* mice compared to the wild-type levels. Moreover, *Min* mice receiving SK-216 at 50 and 100 ppm exhibited significantly reduced total numbers of intestinal polyps, to 64 and 56% of the untreated group value, respectively. Serum TG levels were also decreased by 43% at the dose of 100 ppm. In addition, administration of 50 ppm SK-116, another PAI-1 inhibitor, for 9 weeks similarly reduced serum Pai-1 levels and total numbers of intestinal polyps to 70% of the untreated group value (53). These results indicate that Pai-1 induction associated with hypertriglyceridemia may contribute to intestinal polyp formation with *Apc* deficiency, and PAI-1 could thus be a novel target for colorectal chemopreventive agents (54).

Involvement of leptin in colorectal carcinogenesis

Leptin, a 16-kDa protein produced by the *ob* gene, plays an important role in regulation of food intake and energy balance. Thus, a good correlation of serum leptin levels is observed with the percentage of body fat, values being markedly elevated in obese individuals (55-57). The effects of leptin on peripheral tissue are mediated through binding to its receptor (Ob-R) leading to activation of NFκB, Erk1/2, PI3K/Akt and JAK/STAT signaling (58). All the pathways exert roles in cell survival and proliferation.

The published effects of leptin on the development of colorectal cancer have been somewhat contradictory. In human clinical data, several case–control studies provided evidence that high serum leptin correlated with an elevated risk of colorectal cancer (59, 60), but this was not confirmed by others (61, 62). In the rodent models, leptin exerted no influence on HT-29 colon cancer growth grafted in nude mice or on the intestinal polyp development in *Min* mice, even though plasma leptin levels were 2.4- to 4.3-fold increased by delivery of exogenous leptin (63). In the case of

carcinogen induced colon carcinogenesis models, obese *ob/ob* mice (leptin-deficient mice) and *db/db* mice (Ob-R-deficient mice), it has been reported that intraperitoneal injection of the carcinogen AOM resulted in the development of around 15 colorectal ACF in both strains (64). Another report demonstrated AOM-induced colorectal tumor development, i.e. multiplicity and tumor size, in *ob/ob* and *db/db* mice to be reduced as compared to wild-type mice (65). These findings indicate that leptin acts as a growth factor at some stages in colorectal carcinogenesis. KK-A^y mice are also obese but possess intact leptin and leptin receptors (66). They were established by cross-mating KK mice, Type 2 diabetes model mice, with C57BL/6J-A^y mice (67, 68), which carry the *Agouti* gene (*Ay*), to induce severe hyperphagia, hyperinsulinemia and hyperlipidemia as compared with C57BL/6J mice. C57BL/6J mice are generally used as non-obese, non-diabetic controls compared with KK-A^y mice (69, 70). In this obese KK-A^y mice model, the mice were found to be highly susceptible to induction of ACF, and developed colorectal carcinomas. Furthermore, some of the tumors exhibited cancer cell invasion under the muscular layer of mucosa and remarkable tumor angiogenesis (66). The number of ACF/mouse and tumor/mouse developing in KK-A^y mice (≈70 ACF/mouse and ≈8 tumors/mouse) in response to AOM also

Table 1. Development of colorectal ACF/tumors in obese mice treated with AOM

Mice	AOM treatment (dose, weeks after the last AOM)	No. of ACF / colorectum	No. of tumors / colorectum	Ref.
ob/ob	2-weekly ip (10 mg/kg, 4 weeks)	~35		65
	6-weekly ip (10 mg/kg, 14 weeks)		~2	
db/db	6-weekly ip (10 mg/kg, 14 weeks)		~2	
C57BL	2-weekly ip (10 mg/kg, 4 weeks)	~5		
	6-weekly ip (10 mg/kg, 14 weeks)		~3.5	
KK-A^y	2-weekly ip (200 mg/mouse, 5 weeks)	~70		66
	6-weekly ip (200 mg/mouse, 7 weeks)		~8	
C57BL	2-weekly ip (200 mg/mouse, 5 weeks)	~9		
	6-weekly ip (200 mg/mouse, 7 weeks)		~0.1	
ob/ob	4-weekly ip (5 mg/kg, 100 days)	~15		64
db/db	4-weekly ip (5 mg/kg, 100 days)	~16		
C57BL	4-weekly ip (5 mg/kg, 100 days)	~6		

AOM: azoxymethane; ACF: aberrant crypt foci; ip: intraperitoneal

appeared higher than in other obese mice, *ob/ob* or *db/db* mice (Table 1). The KK-A^y mouse exhibited abdominal obesity, hypertriglyceridemia and hyperinsulinemia at the time-points of colorectal ACF and cancer development. Moreover, serum adipocytokines such as interleukin-6 (IL-6), leptin and Pai-1 were also elevated compared with values for lean C57BL/6J mice. Expression of pro-inflammatory adipocytokine mRNA such as for IL-6, leptin, monocyte chemoattractant protein-1 (MCP-1), Pai-1 and TNF-α were significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Taking together, it would appear that further investigations are required to clarify the role of leptin in colorectal carcinogenesis. Interactions between leptin and insulin may be one of the factors making the story complex. The concept of leptin resistance, INDEX= ln (serum leptin levels/%body fat) (71), might help to clarify the importance of leptin for colorectal and other organ carcinogenesis in the future.

Future aspects

For the present purpose, we have focused on the involvement of three major adipocytokines, adiponectin, PAI-1 and leptin, in colorectal carcinogenesis.. Very recently, it has become clear that some of these hormones may play important roles not only in the stage of progression of colorectal cancer, but also in very early stages of colorectal cancer development. However, cancer prevention/therapies targeting these adipocytokines have yet to be established.

Although improvement of adipocytokine imbalance must be considered as an important goal for prevention of cancer, targeting adipocytokine expression levels for development of cancer chemopreventive and chemotherapeutic agents seems particularly challenging. This is because adipocytokines are expressed in many tissues and play essential roles for biologically indispensable metabolism regulation. Thus, it might be important to develop selective adipocytokine regulating drugs or search for agents from drugs with little side effects, i.e. from the aspect of "drug repositioning".

As an alternative to administration of adipocytokine inducers/activators and suppressors/inhibitors, it is highly desirous that public health measures be taken to increase sportive physical activity and to encourage temperance in daily life so that a meritorious balance of the adipocytokine production is naturally maintained.

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Review Article

Lipoprotein Lipase as a Candidate Target for Cancer Prevention/Therapy

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Epidemiological studies have shown that serum triglyceride (TG) levels are linked with risk of development of cancer, including colorectal and pancreatic cancers, and their precancerous lesions. Thus, it is assumed that serum TG plays an important role in carcinogenesis, and the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may therefore be involved. Dysregulation of LPL has been reported to contribute to many human diseases, such as atherosclerosis, chylomicronaemia, obesity, and type 2 diabetes. Recently, it has been reported that *LPL* gene deficiency, such as due to chromosome 8p22 loss, *LPL* gene polymorphism, and epigenetic changes in its promoter region gene, increases cancer risk, especially in the prostate. In animal experiments, high serum TG levels seem to promote sporadic/carcinogen-induced genesis of colorectal and pancreatic cancers. Interestingly, tumor suppressive effects of LPL inducers, such as PPAR ligands, NO-1886, and indomethacin, have been demonstrated in animal models. Moreover, recent evidence that LPL plays important roles in inflammation and obesity implies that it is an appropriate general target for chemopreventive and chemotherapeutic agents.

1. Introduction

A high-calorie diet and low physical activity, part of the so-called "Westernization" of lifestyle, are associated with elevated incidences of the breast, colon, liver, pancreas, and prostate cancers. Moreover, they are also linked with the risk of obesity, type 2 diabetes, and dyslipidemia. The World Cancer Research Fund and American Institute for Cancer Research have evaluated causal relationships between body fat and cancer and provided strong evidence for roles in such as colorectum and pancreas cancers [1]. In Japan, overweight and obesity (body mass index ≥25) are reported to be associated with cancers of specific organs, such as the colorectum (male), postmenopausal breast (female), and the liver in individuals positive for hepatitis C virus infection [2–4].

Greater body fatness is a major risk factor for the metabolic syndrome, which presents as a combination of symptoms, such as dyslipidemia (elevated triglyceride (TG) levels or low high-density lipoprotein (HDL) cholesterol), elevated blood pressure, and elevated fasting glucose levels. Hypertriglyceridemia is associated with the risk of colon cancer in Japanese men (HR = 1.71) and being overweight with the risk of breast cancer (HR = 1.75) [5]. In addition, most epidemiological studies, including our own, have consistently showed that serum TG levels are associated with the risk of colorectal adenoma, a precursor lesion of colorectal cancer [6–11]. Thus, it is assumed that serum TG could play an important role in carcinogenesis and that the key enzyme lipoprotein lipase (LPL), which catalyzes the hydrolysis of plasma TG, may also be involved. In this paper, we focus on the roles of LPL in cancer development and further discussed possible approaches to cancer prevention/therapy.

2. Function, Structure, and Gene Regulation of LPL

2.1. Functions and Structure of LPL. LPL plays an important role in lipid metabolism as an enzyme responsible for hydrolysis of the TG component in circulating chylomicrons and very-low-density lipoprotein (VLDL) via binding with apolipoprotein C2 [12, 13]. Thus, lowering or deficiency of LPL expression is associated with hyperlipidemia [14, 15]. The LPL enzyme itself is composed of two structurally

distinct regions. The amino-terminal domain is responsible for catalysis with a catalytic center formed by three amino acids (Ser^{132}, Asp^{156}, and His^{241}). The carboxy-terminal domain of LPL is required for its binding to the lipoprotein substrate [3, 16–18].

2.2. LPL Gene Expression and Its Regulation. The human *LPL* gene is located on chromosome 8p22 and composed of 10 exons [19]. LPL is ubiquitously expressed in the whole body, but especially in the adipose tissue and the skeletal muscle [20, 21] and is regulated by hormonal and inflammatory stimuli, such as insulin [22, 23], glucocorticoid [24, 25], adrenaline [26], tumor necrosis factor (TNF)-α [27, 28], transforming growth factor (TGF)-β [29], and interleukin (IL)-1β [27].

The expression of LPL is controlled transcriptionally and posttranscriptionally. Basal promoter activity has been shown to be regulated by Oct-1 and the NF-Y binding motifs [30, 31], and the 5′-CCTCCCCC-3′ motif, which interacts with Sp1 and Sp3 [32]. Induction of *LPL* gene transcription is mediated by the peroxisome proliferator response element (PPRE) and the responsible element which binds to sterol regulatory element-binding protein (SREBP) [33, 34]. The effect of insulin on *LPL* expression is an example of posttranscriptional control, the hormone being suggested to increase *LPL* mRNA levels via mRNA stabilization [23, 35].

3. Relationship between LPL and Cancer: Human Studies

3.1. Loss of LPL and Resultant Common Disease. LPL has been reported to play key roles in many human diseases, such as atherosclerosis, obesity, type 2 diabetes, chylomicronaemia, Alzheimer's disease, and cachexia [15]. Especially, *LPL* gene deficiency is the cause of type I hyperlipoproteinemia (familial hyperchylomicronemia) [36]. Homozygous deficiency of *LPL* in humans is rare, but heterozygous deficiency is observed in around 3% of people with various ethnic backgrounds [37, 38]. Although these individuals have elevated serum levels of TG and decreased HDL cholesterol [39], it is not clear whether they are at increased risk of atherosclerosis, ischemic heart disease, type 2 diabetes, and cancer. There is a report that the *LPL* S447X mutation is associated with a higher risk of pancreatic calcification and steatorrhea in hyperlipidemic pancreatitis [40]. Since LPL provides fatty acids to the tissues and fatty acids evoke insulin resistance, *LPL* gene deficiency could affect glucose metabolism. However, whether heterozygous *LPL* deficiency reduces plasma glucose levels or not is still controversial. One paper described reduction of plasma glucose levels, but two others observed no effects as compared with LPL intact humans [41–43]. On the other hand, it has been reported that patients with poorly controlled diabetes frequently have dyslipidemia due to defects in LPL enzyme activity [44].

3.2. Effects of Chromosome 8p22 Loss and LPL Gene Polymorphisms on Cancer Risk. Alteration in genomic DNA, such as point mutations and deletions/amplifications or epigenetic changes such as CpG island hypermethylation and histone modification, can induce abnormal gene expression, which in the case of tumor suppressor genes or oncogenes could eventually lead to carcinogenesis. The human *LPL* gene has been mapped to chromosome 8p22 and previous studies on loss of heterozygosity (LOH) in colorectal tumors suggested that a putative tumor suppressor gene may lie within the short arm of chromosome 8, that is, 8p22-p21.3. Loss of 8p23.1-22 is also reported to be an important stage in initiation or promotion of hepatocellular carcinoma development and may also be the most frequent chromosomal alteration in prostate cancer [45]. It has been found that deletion of *LPL* is observed in 68% (52/76) of localized prostate cancers by FISH analysis [46]. It has further been reported that chromosomal region 8p23.1-8p21.1 may harbor one or more important prostate-cancer-susceptible loci based on linkage analyses in 159 hereditary prostate cancer families [47, 48]. To date, several new candidate cancer-susceptible genes have been cloned to 8p22, such as *deleted in breast cancer 2* (*DBC2*), *leucine zipper tumor suppressor 1* (*LZTS1*), *deleted in liver cancer 1* (*DLC1*), and *mitochondrial tumor suppressor 1* (*MTUS1*) [49–52]. Thus, cancer-susceptible genes mapped close to the *LPL* gene could be affected by *LPL* gene deletion, and exert combined effects in promoting carcinogenesis.

Moreover, an *LPL* Ser447stop polymorphism has been shown to be associated with prostate cancer risk [53] and the *LPL* gene is commonly methylated in prostate tumors [54]. *LPL* promoter CpG island methylation has been revealed in 45% of LPL-deleted tumors and in 22% of LPL-retaining tumors [54]. Biallelic inactivation of *LPL* by chromosomal deletion and promoter methylation may thus contribute to prostate tumorigenesis, but information is lacking regarding pancreatic cancer.

4. Relationship between LPL and Cancer: Animal Studies

4.1. Dyslipidemia Observed in Cancer-High-Susceptibility Animal Models. Elevated serum TG has been shown to promote carcinogen-induced colon carcinogenesis, and rats with hypertriglyceridemia such as the Zucker obese and Nagase analbuminemic strains and F344 rats fed a high-fat diet are all known to be more sensitive to carcinogen treatments than rats with normal serum lipid levels [55–57].

In the case of mice, the Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$) [58] and Min (C57BL/6-$Apc^{Min/+}$) animal models of human familial adenomatous polyposis (FAP) feature development of large numbers of intestinal polyps and hypertriglyceridemia [59, 60]. Although no significant differences between Apc^{1309} mice and wild-type mice were observed at 6 weeks of age, the average serum TG value in the former at 12 weeks was obviously increased almost 10-fold (~600 mg/dL) over that at 6 weeks. Similar increase of TG levels (~400 mg/dL) was observed in Min mice at 15 weeks compared to 8 weeks of age (Table 1). Along with TG elevation, mRNA levels of *LPL* in the liver and small intestine of Apc^{1309} and Min mice were suppressed. Of note, other lipogenic genes, such as *FAS* and *stearoyl-CoA*

Table 1: Summary of animal models with dyslipidemia and cancer high susceptibility.

Animal	Strain	Age (week-old)	Serum TG (mg/dL)	Treatment	Tumor	Reference
Mouse	Apc^{1309} (C57BL/6J$^{Apc/Apc\Delta 1309}$)	12	~600	—	Intestinal adenoma	[59]
	Min (C57BL/6-$Apc^{Min/+}$)	15	~400	—	Intestinal adenoma	[59, 60]
	KK-A^y	19	481	AOM	Colon cancer	[61]
	ICR	20	159	AOM + DSS	Colon cancer	[62]
Syrian golden hamster	—	6	300	BOP	Pancreatic cancer	[63]

Table 2: Summary of tumor suppressive effects of LPL inducers in animal models.

Agent	Dose	Animal model	Value to the untreated control group	Reference
Pioglitazone	200 ppm	Apc^{1309}	67%	[59]
	1600 ppm	Min	9%	[60]
	800 ppm	BOP-treated hamster	40%	[63]
NO-1886	800 ppm	Min	42%	[65]
Indomethacin	10 ppm	Min	25%	[66]

desaturase-1, β-oxidation genes like *acyl-CoA oxidase* and *carnitine palmitoyl transferase 1*, and gluconeogenesis genes, exemplified by *phosphoenolpyruvate carboxykinase*, demonstrated no variation from wild-type mouse expression.

Obese KK-A^y mice were found to be highly susceptible to azoxymethane- (AOM-) induced colorectal aberrant crypt foci (ACF) and colorectal carcinoma development compared to lean C57BL/6J mice [61]. Surprisingly, colorectal carcinomas developed within a very short-term period, 19 weeks, after AOM injection. The number of total ACF in KK-A^y mice was around 70/mouse and almost 8 times higher than that in lean C57BL/6J mice. The incidences of adenomas and adenocarcinoma were 84% and 88%, respectively, in KK-A^y mice, far higher than the 8% and 4% in C57BL/6J values. KK-A^y mice exhibit abdominal obesity, hypertriglyceridemia, and hyperinsulinemia at the time of ACF and tumor development. At 13 weeks of age, the average serum levels of TG, total cholesterol, and free fatty acids of KK-A^y mice undergoing AOM treatment were 484.1 mg/dL, 101.6 mg/dL, and 1,796 mEq/L, respectively (Table 1). It is interesting that hepatic *LPL* mRNA levels were also suppressed in KK-A^y mice compared with C57BL/6J mice. Moreover, serum proinflammatory adipocytokines, such as IL-6, leptin, and plasminogen activator inhibitor-1 (Pai-1), were elevated. Importantly, expression of proinflammatory adipocytokine mRNAs such as for IL-6, leptin, monocyte chemotactic protein (MCP)-1, Pai-1 and TNF-α was significantly increased in the visceral fat tissue; in contrast, that for adiponectin was decreased.

Tanaka et al. have developed a novel colitis-related colorectal carcinogenesis model, using AOM plus dextran sodium sulfate (DSS), a colitis-inducing agent [64]. In this model (AOM + 2% DSS in ICR mice), numerous colorectal adenocarcinomas occur within a short-term period and the serum TG levels demonstrate increase to about 134, 175 and 159 mg/dL at 5, 10, and 20 weeks, respectively [62] (Table 1).

Injection of *N*-nitrosobis(2-oxopropyl)amine (BOP) into Syrian golden hamsters is known to induce pancreatic ductal adenocarcinomas, with a histology very similar to typical human pancreatic ductal adenocarcinomas. Moreover, associated genetic mutations, that is, K-*ras* point mutations and *p16* aberrant methylation/homozygous deletions, are found in common in both hamster and human lesions. Interestingly, Syrian golden hamsters exhibit a hypertriglyceridemic state, almost 300 mg/dL at 6 weeks of age, even when not fed a high-fat diet [63] (Table 1). Also, in the case of this animal model, a low activity of LPL could be one of the causes of hypertriglyceridemia, activity of this enzyme in the liver being only 20% and 30%, respectively, of the values in C57BL mice and F344 rats.

5. Tumor Suppressive Effects of LPL Inducers

Pioglitazone, {(±)-5-[4-[2-(5-ethyl-2-pyridyl)ethoxy]benzyl]thiazolidine-2,4-dione monohydrochloride}, is a potent peroxisome proliferator-activated receptor (PPAR)γ ligand with a weak binding affinity for PPARα. In the promoter region of the *LPL* gene, there exists a PPRE, and pioglitazone treatment successfully induced LPL expression in the liver and intestinal epithelial cells in *Apc*-deficient mice. The total numbers of polyps in the groups treated with 100 and 200 ppm pioglitazone in the Apc^{1309} were reduced to 67% of the value in the untreated control group [59] (Table 2). With another *Apc*-deficient model, Min mice given 100–1600 ppm pioglitazone for 14 weeks showed decrease of intestinal polyps to 63–9% of the control number [60] (Table 2 and Figure 1).

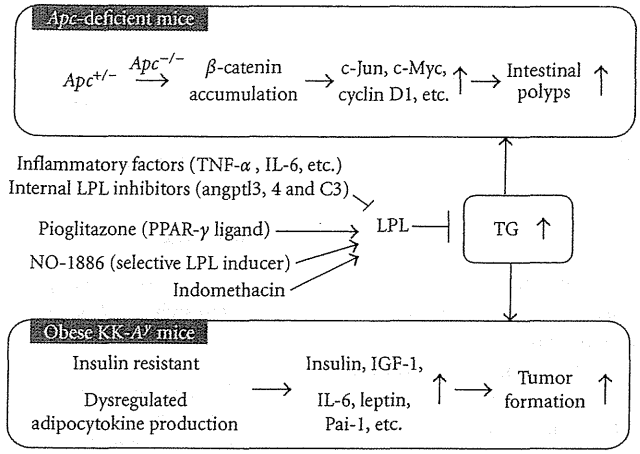

FIGURE 1: Involvement of triglycerides in animal intestinal carcinogenesis models. Angptl-3,4: angiopoietin-like protein-3,4; IGF-1: insulin like growth factor-1; IL-6: interleukine-6; LPL: lipoprotein lipase; Pai-1: plasminogen activator inhibitor-1; PPAR: peroxisome proliferator-activated receptor; TG: triglyceride; TNF-α: tumor necrosis factor-α.

Pioglitazone possesses other functions rather than just simply inducing LPL, such as causing cell growth arrest and apoptosis. Thus, data regarding LPL selective inducers are necessary for determining the relationship between hypertriglyceridemia and intestinal carcinogenesis. NO-1886, 4-[(4-bromo-2-cyanophenyl)carbamoyl] benzylphosphonate, chemically synthesized at Otsuka Pharmaceutical Factory [67] is one useful tool for clarifying this issue. Using a reporter gene assay, NO-1886 demonstrated no PPAR agonistic activity, unlike bezafibrate and pioglitazone [68].

Administration of 400 and 800 ppm NO-1886 also significantly decreased the total number of intestinal polyps to 48% and 42% of the untreated control value, respectively, in Min mice, along with causing marked increase in *LPL* mRNA levels in the liver and the small intestine. Moreover, treatment with NO-1886 also significantly decreased the numbers of colon polyps [65] (Table 2, Figure 1).

In the case of BOP-treated hamsters, pioglitazone has been demonstrated to improve hyperlipidemia and suppress ductal adenocarcinoma development. The incidences of ductal adenocarcinoma in the BOP plus 800 ppm pioglitazone and BOP alone groups were 38% and 80%, and the multiplicities were 0.55 and 1.37, respectively [63] (Table 2). Expression levels of hepatic LPL mRNA were elevated by treatment with 800 ppm pioglitazone. Moreover, quantitative real-time RT-PCR assays demonstrated almost 1.7-fold higher mRNA levels of LPL than that of pioglitazone-nontreated hamsters.

Indomethacin is a conventional nonsteroidal anti-inflammatory drug which has long been clinically employed to improve inflammation. It has demonstrated potent chemopreventive activity against intestinal tumor development in animal models, and a clinical trial in FAP patients also showed reduction in intestinal polyp development [69, 70]. We earlier reported that indomethacin suppresses intestinal polyp formation in Min mice together with ameliorating the hyperlipidemic state by regulating LPL, other lipid metabolic factors and inflammatory pathways [66]. Reduction of serum TG levels was 90% in Min mice with 10 ppm indomethacin treatment and higher than that with 400 ppm pioglitazone (83%) observed in our other previous study [59, 60]. The PPARγ agonistic activity of indomethacin is reported to be 50 times weaker than that of troglitazone, a well-established PPARγ agonist [71]. These results indicate that functions other than agonistic activity of indomethacin are responsible for its strong lipid-lowering effects (Figure 1).

6. Involvement of LPL in Inflammation, Obesity, and Others

6.1. LPL and Inflammation and Apoptosis. In addition to the lipid modifying function of LPL, two different mechanisms might be involved in *LPL* influence on carcinogenesis. The first involves anti-inflammatory action of LPL. It has been reported that LPL suppresses TNF-α- and interferon (IFN)-γ-evoked inflammation-related gene expression in endothelial cells through inactivation of transcription factor nuclear factor kappa B (NF-κB) [72]. Conversely, TNF-α, IFN-γ, IL-1β, IL-6, and leukemia inhibitory factor (LIF) decrease LPL activity.

It is well known that cyclooxygenase-2 (COX-2) is markedly elevated in human colon cancers, in AOM-treated rats, and in intestinal polyps of *Apc*-deficient mice. COX-2 is in fact thought to play important roles in both cancer cell proliferation and angiogenesis. Experiments conducted to clarify the mechanisms of NO-1886 effects on colon carcinogenesis revealed that the expression levels of mRNA for COX-2, in DLD-1 human colon cancer cells, were reduced under conditions of TGFα stimulation. On the other hand, there was no obvious change in the mRNA levels for COX-1 and inducible nitric oxide synthase (iNOS). The results obtained by RT-PCR analysis were also confirmed by

β-gal reporter gene assay in DLD-1 cells [65]. Consistent with the *in vitro* data, administration of 400 and 800 ppm NO-1886 reduced COX-2 mRNA levels in normal parts of small intestine of Min mice at 20 weeks of age [65]. In addition, NO-1886 ameliorates and induces regression of experimental steatohepatitis through increasing LPL activation and suppression of proinflammatory agents, such as TNF-α, IL-6, and COX-2 [73]. Recently, mice lacking *angiopoietin-like protein family 4* (*Angptl4*), which is the inhibitor of LPL, showed a severe and lethal phenotype characterized by fibrinopurulent peritonitis, ascites, intestinal fibrosis, and cachexia in response to a saturated fat diet [74].

The second mechanism is modification of the apoptosis pathway by LPL activation. Phosphatase type 2Cβ activation by unsaturated fatty acids has been demonstrated to induce apoptosis [75]. Unlike ester bodies of fatty acids, free fatty acids have cytotoxic effects *in vitro* and the products produced by hydrolysis of plasma TG may be implicated in such an apoptotic effect.

6.2. LPL and Obesity. Given the importance of LPL for lipid metabolism, its activity would be expected to be intimately involved in obesity effects and development of the metabolic syndrome. A large number of studies in rodents and humans have revealed that obesity results in increased LPL activity in adipose tissue [15, 35, 76–78]. Interestingly, LPL is regulated in opposite directions in adipose tissue and muscle. Feeding increases adipose LPL activity with a corresponding decrease in muscle LPL activity [35, 79]. Exercise stimulates LPL activity in the muscle and leads to increase fatty acid oxidation [80]. In an animal study, NO-1886 suppressed high-fat diet-induced fat accumulation in rats due to the increase of muscle LPL activity [81].

7. Conclusion

Targeting LPL activity or expression levels for development of reagents against cancer seems particularly challenging, because LPL is expressed ubiquitously and plays essential roles in maintaining homeostasis in the body. Data from LPL homozygous knockout mice, which die within one day of birth, underline its importance. However, appropriate suppression of serum TG levels could be achieved by using drugs, even if the number of selective inducers of LPL is limited. Thus, it might be important to develop selective LPL inducers or search for agents focusing on the aspect of "drug repositioning" to obtain the tools for investigating correlation between LPL and cancer. It should be borne in mind that LPL is inhibited by intrinsic factors, such as angptl3, angptl4, and C3 (Figure 1). These could clearly be candidate target molecules for development of LPL inducers. Considering that LPL activity has impact on obesity and metabolic syndrome, its targeting may also affect the regulation of adipocytokines, which may also be involved in carcinogenesis. Further investigations are warranted to clarify the importance of LPL and to accumulate evidence as to the worthiness as a target for cancer chemopreventive and chemotherapeutic agents.

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第3次対がん総合戦略研究事業

がん化学予防剤の開発に関する基礎及び臨床研究

平成22年度～25年度　総合研究報告書

Ⅰ．　総合研究報告

Ⅱ．研究成果の刊行に関する一覧表

（　２／５　冊）

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平成２６（２０１４）年３月

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Non-invasive X-ray Micro-computed Tomographic Evaluation of Indomethacin on Urethane-induced Lung Carcinogenesis in Mice

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Abstract. *Background: Lung cancer is the leading cause of cancer-related death worldwide. We previously reported that respiration-gated X-ray micro-computed tomography (micro-CT) is a useful tool for analyzing lung tumor development in animal models. Materials and Methods: Lung tumors were induced by a single intraperitoneal injection (250 mg/kg) of urethane in male A/J mice, followed by indomethacin treatment at 5 ppm in the diet. The mice were scanned by micro-CT every 4 weeks from 10 to 26 weeks after urethane administration. Results: Total incidence and multiplicity of lung tumors were not significantly reduced by indomethacin treatment, as compared with untreated mice. However, the incidence of adenocarcinoma tended to be reduced by indomethacin treatment. Moreover, the size of lung tumors, especially adenomas, was suppressed by indomethacin treatment. Micro-CT analysis revealed that indomethacin effectively suppressed tumor development after urethane treatment for 10 weeks. Conclusion: These findings indicate that indomethacin suppresses lung carcinogenesis in mice and micro-CT is a useful non-invasive imaging approach for evaluating the characteristics and suppression of lung tumors in mice treated with cancer chemopreventive agents.*

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Lung cancer is the leading cause of cancer-related death worldwide (1), and reducing tobacco use and exposure to environmental carcinogens are effective ways to prevent lung carcinogenesis. The recent development of high-resolution computed tomography is able to identify small nodules in the lungs, including focal ground-glass opacities, which need to be followed in cancer check-ups. Thus, development of effective methods for preventing lung carcinogenesis is an urgent task.

Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin and aspirin, are reported to be useful chemopreventive agents for colorectal cancer, as demonstrated by experimental, epidemiological and clinical studies (2-6). NSAIDs, including indomethacin have also been shown to be useful candidate chemopreventive agents for lung tumors (7, 8). It has already been reported that indomethacin reduces the number of urethane-induced lung tumors in A/J mice (9). Indomethacin is a conventional NSAID, which has long been clinically employed to target inflammation. The molecular mechanisms underlying its protective effects are considered to be mainly due to inhibition of the activity of cyclooxygenase-1 (COX-1) and COX-2, key enzymes of prostanoid synthesis (10).

We recently applied X-ray micro-computed tomography (micro-CT) to detect lung space occupied lesions (SOLs) induced by a single intraperitoneal injection (250 mg/kg

BW) of urethane in male A/J mice, from 10 to 30 weeks after exposure to the carcinogen, and provided evidence that micro-CT is a useful non-invasive imaging approach for evaluating the characteristics and growth of lung tumors in mice (11). Our results also indicated that tumors grew at markedly varying speeds, and reflect histopatholgical findings after autopsy. Furthermore, these results indicate that micro-CT is also useful for evaluating lung tumor regression, induced by cancer chemopreventive agents.

In the present study, pre-neoplastic and neoplastic lesions (hyperplasia, adenoma and adenocarcinoma) were induced in the lungs of male A/J mice by a single intraperitoneal injection of urethane with or without indomethacin treatment at 5 ppm in the diet to evaluate its chemopreventive effects. In addition, lung SOL development was monitored periodically using respiration-gated micro-CT.

Materials and Methods

Animals. A/J Jms Slc mice, 5-week-old males, were purchased from Japan SLC Inc. (Hamamatsu, Japan) and five mice each were housed in a plastic cage with wood chip bedding in an air-conditioned animal room maintained at 24±2°C and 60±5% relative humidity, with a 12 h light-dark cycle. Basal diet (AIN-76A; CLEA Japan, Inc., Japan) and water were available *ad libitum* throughout the experiment.

Experimental protocol for A/J mice treated with indomethacin. At 6 weeks of age, mice (n=9) were treated with a single intraperitoneal injection of urethane (250 mg/kg; Sigma, St Louis, MO, USA) in 0.9% NaCl saline. Control mice (n=5) were given a single saline intraperitoneal injection. At the same time administration of indomethecin was started. Indomethacin was purchased from Sigma Chemical Co. (St Louis, MO, USA) and well-mixed at concentrations of 5 ppm with the basal diet. The dosage of indomethacin was determined by a previous report and our experiment (9, 12). The mice were scanned by micro-CT every 4 weeks from 10 to 26 weeks after urethane or control vehicle (0.9% NaCl saline) injection. The experiments were conducted according to the Guidelines for Animal Experiments in the National Cancer Center of the Committee for Ethics of Animal Experimentation of the National Cancer Center.

Micro-CT scan procedure. All mice were anesthetized with isoflurane (Dainippon Sumitomo Pharmaceutical Co., Osaka, Japan) and maintained anesthesia was achieved with a mixture of isoflurane and room air delivered during the scanning with micro-CT. Each mouse was placed on its back on an animal bed for micro-CT scanning, banded across the chest area, and a sensor for detecting respiration was placed on the abdomen. The X-ray scanning time point was set at 1,200 ms after expiration.

For scanning, a new cone-beam micro-CT scanner (eXplore Locus; General Electric Healthcare, London, Ontario, Canada) was used. The scan parameters that are consistent with gated *in vivo* scan acquisition include: 80 kV peak, 450 μA, 400 ms per frame, 0.5 degree at angle of increment and 720 views. The measured in-air radiation at iso-center was 240 mGy. Three-dimensional images obtained from axial, sagittal, coronal and oblique micro-CT images were reconstructed using MicroView (General Electric Healthcare).

Table I. *Multiplicity of lung SOLs in A/J Mice Treated with or without Indomethacin Assessed by Micro-CT.*

Indomethacin (ppm)	No. of mice	No. of SOLs/mouse				
		10	14	18	22	26 weeks
0	9	4.2±2.2	5.6±2.2	8.8±2.2	9.1±2.7	10.0±3.0
5	9	3.9±2.3	5.8±2.2	7.1±2.4	7.6±2.6	8.3±3.2

Data are mean±SD. SOL, space occupied lesion.

Histopathological examination. The mice were sacrificed 26 weeks after urethane administration and major organs, liver, kidneys and spleen, were weighed before fixation in 10% buffered formalin. Lungs were inflated for this purpose and lung SOLs, detected by a stereoscopic microscope, were embedded in paraffin blocks and sectioned at 3 μm for placement on slides and staining with hematoxylin and eosin for histopathological evaluation. Lung SOLs were diagnosed according to the criteria of the "International Classification of Rodent Tumors, The Mouse" (13) by a pathologist.

Human lung tissue samples. A total of 23 lung tissue samples were obtained from patients who underwent lobectomy at the National Cancer Center Hospital from 2006 to 2008. The paraffin-embedded sample stocks were used for integrin immunohistochemical staining. The samples include normal lung tissue (n=1), atypical adenomatous hyperplasia (n=11) and localized tumors with a lepidic growth pattern and alveolar collapse (Noguchi type B, n=11) (14), which were diagnosed by a pathologist. The study protocol was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Immunohistochemical staining. The sections used for histopathological evaluation in mice and humans were also used for immunohistochemical examination with the avidin–biotin complex immunoperoxidase technique. Polyclonal goat anti-COX-2 antibody (M-20) and polyclonal rabbit anti-vascular endothelial growth factor (VEGF) antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:100 dilution were used. As a secondary antibody, anti-goat IgG, biotinylated and absorbed with rat serum (Vector Laboratories, Burlingame, CA, USA), was employed at 1:200 dilution. Staining was performed using avidin–biotin reagents (Vectastain ABC reagents; Vector Laboratories), 3,3'-diaminobenzidine and hydrogen peroxide, and the sections were counterstained with hematoxylin to facilitate orientation. As negative controls, consecutive sections were immunostained without exposure to the primary antibody.

Statistical analysis. The significance of differences in the multiplicity of urethane-induced mouse lung SOLs was analyzed using the Student's *t*-test, and statistical analysis for the number of SOLs which had more than doubled in diameter was performed with the χ^2 test. Differences were considered to be statistically significant with *p*-values of less than 0.05.

Table II. *Incidence and multiplicity of lung SOLs in A/J mice treated with or without indomethacin, assessed histopathologically.*

Indomethacin (ppm)	No. of mice	Hyperplasias		Adenomas (Ad)		Adenocarcinomas (Ca)		Total SOLs	Ad+Ca
		Incidence	Multiplicity	Incidence	Multiplicity	Incidence	Multiplicity	Multiplicity	Multiplicity
0	9	9 (100)	4.0±2.1	9 (100)	5.4±2.1	4 (44)	0.9±1.2	10.3±2.6	6.3±2.6
5	9	9 (100)	3.1±2.8	9 (100)	5.3±2.3	1 (11)	0.1±0.3^{+}	8.6±3.7	5.4±2.4

Data are mean±SD. Numbers in parentheses are percentages of mice with urethane-induced lung SOLs. ^{+}p=0.08 *vs.* 0 ppm.

Results

Incidence and multiplicity of lung SOLs assessed by micro-CT and histopathological analysis. The lung SOLs induced by urethane were easily distinguished from surrounding tissues in the micro-CT images. Reconstructed three-dimensional images were useful to differentiate the masses (globular) and blood vessels (tube structure) in lungs, even though both have a similar X-ray absorption. The smallest detectable SOL was approximately 0.5 mm in diameter. The incidence of SOLs detected by micro-CT was 100%, (10-26 weeks after urethane treatment). The number of SOLs/mouse (multiplicity) detected by micro-CT increased from 10 to 26 weeks after urethane treatment, as shown in Table I. The multiplicity of lung SOLs at 26 weeks after urethane treatment was 10.0±3.0 (mean±SD). With indomethacin treatment, the multiplicity of lung SOLs, determined by micro-CT at the end of the experiment, was reduced by approximately 20% as compared with the untreated mice (Table I).

Table II shows the incidence and multiplicity of lung SOLs at the end of experimental period, as determined by histopathological analysis. The number of total SOLs (10.3±2.6) was similar to that detected by micro-CT. The incidence of hyperplasia (bronchiolo-alveolar hyperplasia) and adenoma (bronchiolo-alveolar adenoma) was 100%, and that of adenocarcinoma (bronchiolo-alveolar adenocarcinoma) was 44%. No spontaneous tumors were observed in the A/J mice without urethane treatment. The actual number of lung SOLs examined by histopathological analysis was: eight adenocarcinomas in the indomethacin-untreated group, and one adenocarcinoma in the indomethacin-treated group. Similar numbers of hyperplasia and adenoma were detected in both indomethacin-treated and untreated groups. The multiplicity of adenocarcinoma averaged 0.9 in the indomethacin-untreated mice, and tended to be reduced to 0.1 (p=0.08) in the indomethacin-treated mice (Table II).

Change of number of lung SOLs by periodic micro-CT analysis. Periodic micro-CT analysis of urethane-induced lung SOLs in living mice revealed that the total number of SOLs started to differ between groups from 18 weeks after urethane injection (Figure 1). Consistent with previous work (9), the percentage of reduction was almost the same throughout the experiment. Thus, the number of newly-developed SOLs within 4 weeks, *i.e.* newly-detected SOLs by micro-CT, in mice was counted successively, and are presented in Figure 1. Moreover, histopathological analysis revealed that tumors diagnosed as adenocarcinoma at the end of the experiment existed as SOLs in CT images from the early experimental periods: three tumors at 14 weeks and one tumor at 18 weeks. Interestingly, the number of newly-developed lung SOLs in the untreated group started to

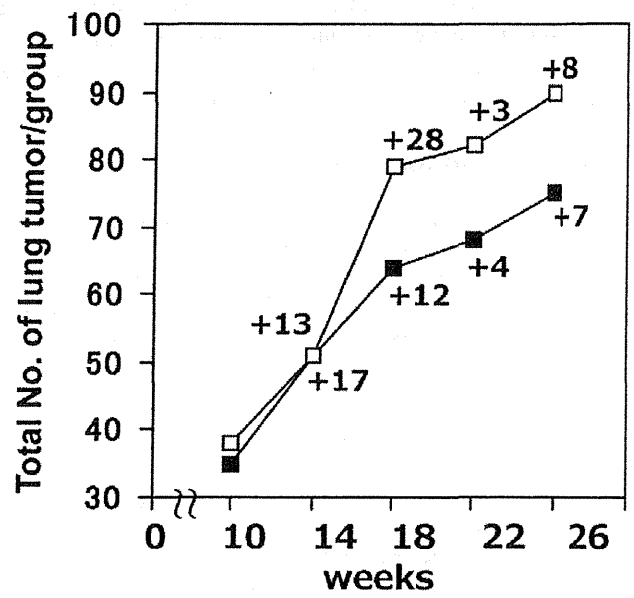

Figure 1. *Total number of lung space occupied lesions (SOLs) assessed by micro-computed tomography (CT). Increase of total lung SOLs from 10 to 26 weeks after urethane injection, detected by micro-CT, is shown. Open squares represent the control untreated group and closed squares indicate the indomethacin-treated group. The number of newly-detected SOLs in each group from 4 weeks is shown above or below the respective line.*

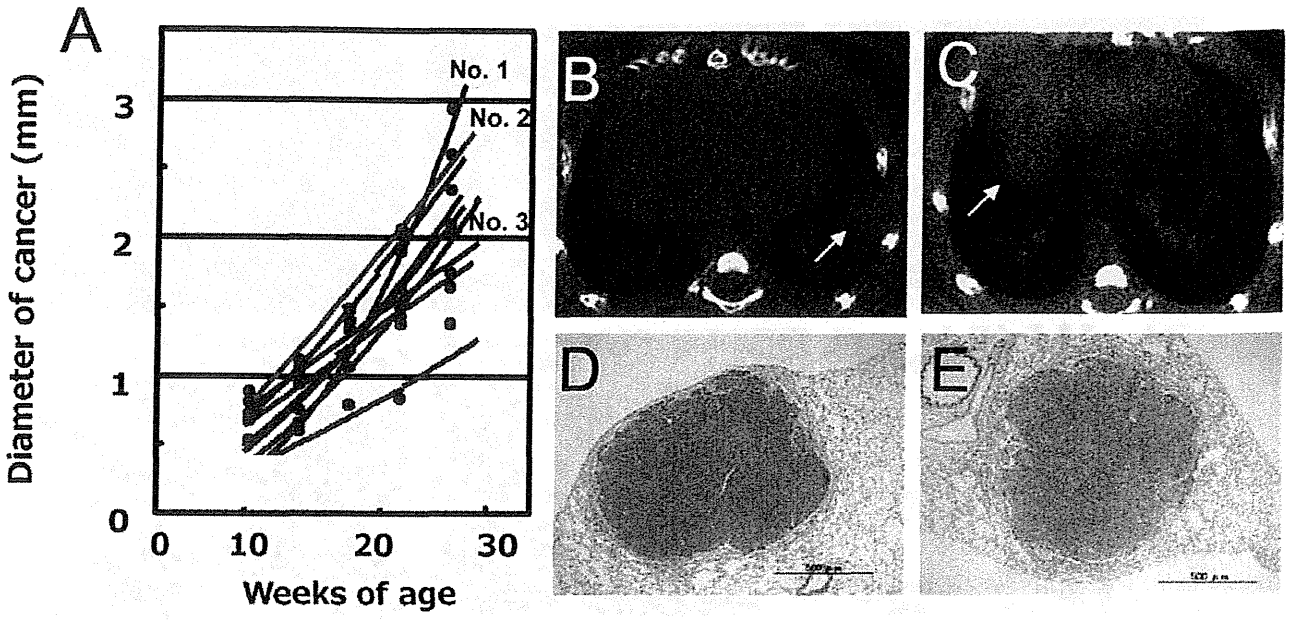

Figure 2. *Increase of lung adenocarcinoma diameters in A/J mice by axial micro-computed tomography (CT) images and histopathological hematoxylin and eosin (HE) staining. A: Growth curves of nine adenocarcinomas are shown. Each tumor scanned by micro-CT was reconstructed into three-dimensional images (axial, sagittal, coronal and oblique) and maximum diameters were measured periodically. The red line shows the growth of adenocarcinoma in the indomethacin-treated group. The blue line shows that of the untreated group. B: Micro-CT images of the most aggressive lung adenocarcinoma (curve no. 1 in A). C: Micro-CT images of lung adenocarcinoma in the indomethacin-treated group (curve no.2 in A). D: Histopathology of the lung space occupied lesion (SOL) shown in B. E: Histopathology of tumor shown in C. (D and E: bar=500 μm). Tumors observed in the lung are shown by arrows.*

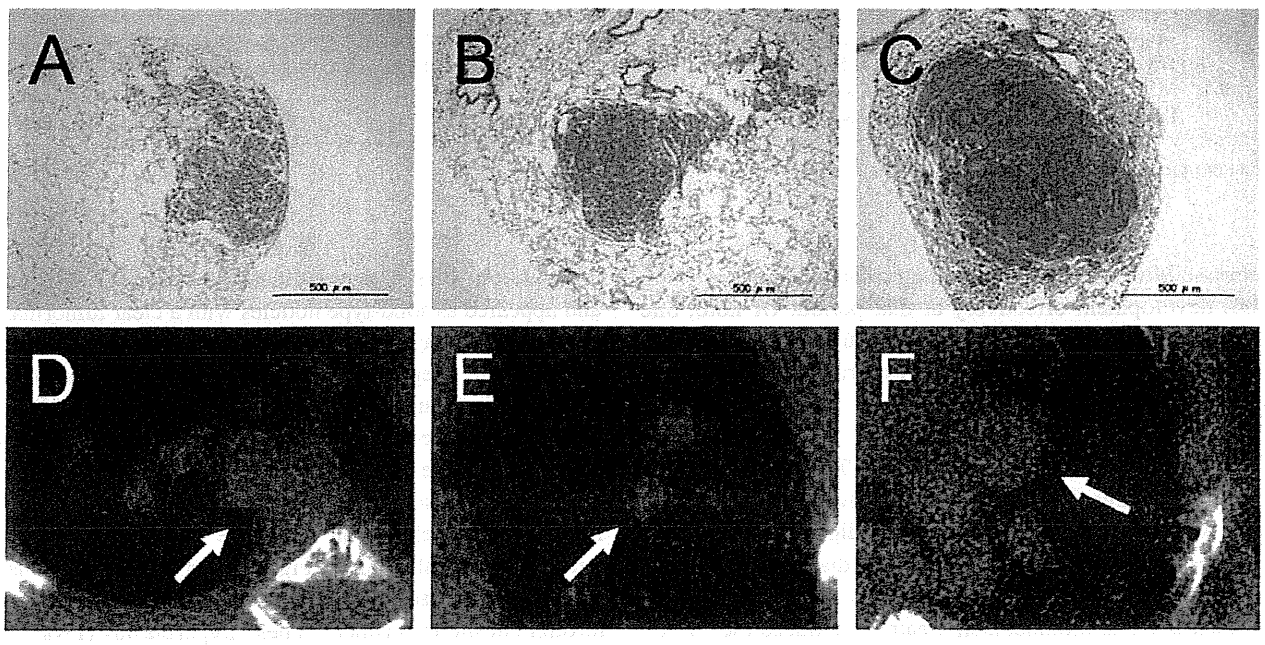

Figure 3. *Virtual in vivo micro-computed tomography (CT) images of lung space occupied lesion (SOL) and histopathological findings. Axial micro-CT images of the thorax of a mouse at the end of the experiment are shown. Histopathology of representative hyperplasia (A), adenoma (B) and adenocarcinoma (C) observed in urethane-treated mouse (all indomethacin-untreated mice); bar=500 μm. Micro-CT images representing hyperplasia (D), adenoma (E) and adenocarcinoma (F). Tumors observed in the lung are shown by arrows.*